REVIEW

Human circulating monocytes as multipotential progenitors

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Abstract. Circulating monocytes are believed to be committed precursors for phagocytes, such as macrophages and dendritic cells. Recently, we have reported a primitive human cell population called monocyte-derived multipotential cells (MOMC), which has a fibroblast-like morphology and a unique phenotype positive for CD14, CD45, CD34, and type I collagen. This novel cell type exhibits mixed morphologic and phenotypic features of monocytes, endothelial cells, and mesenchymal cells. MOMC are derived from circulating $CD14^+$ monocytes, and their differentiation requires binding to fibronectin and exposure to one or more soluble factors derived from peripheral blood $CD14^-$ cells. MOMC contain progenitors with capacity to differentiate into a variety of non-phagocytes, including bone, cartilage, fat, skeletal and cardiac muscle, neuron, and endothelium. Recent studies by others have also described several distinct human cell populations that are originated from circulating monocytes and have capacity to differentiate into non-phagocytes. These observations together indicate that circulating monocytes are more multipotential than previously thought. In addition, cell transplantation therapies using circulating monocytes are a potential approach for tissue regeneration. (Keio J Med 56 (2) : 41 – 47, June 2007)

Key words: differentiation, monocytes, progenitor, regeneration

Introduction

Circulating $CD14^+$ monocytes are originated from hematopoietic stem cells in the bone marrow and consist of 5 to 10% of circulating white blood cells in humans. They are heterogeneous population in terms of surface markers, phagocytic capacity, and differentiation potentials, but are committed precursors in transit from the bone marrow to ultimate sites of activity.[1] Circulating monocytes have capacity to differentiate into a variety of phagocytes, including macrophages, dendritic cells, osteoclasts, microglia in the central nervous system, and Kupffer cells in the liver.[1–4] Until recently, it has been believed that the differentiation potential of monocytes is restricted to cells possessing phagocytic capacity, which function as phagocytes and/or specialized antigen-presenting cells. However, recent accumulating evidence indicates that circulating monocytes have potential to differentiate into variety of cell types other than phagocytes. Our recent discovery of a primitive cell population termed monocyte-derived multipotential cells (MOMC) supports a concept of the multipotential nature of circulating monocytes.[5] This review summarizes current understandings of non-phagocytic differentiation potentials of human circulating $CD14^+$ monocytes.

Identification of MOMC

MOMC, previously called monocyte-derived mesenchymal progenitors (MOMP), are a human cell population with a fibroblast-like morphology, and have a unique phenotype positive for CD14, CD45, CD34, and type I collagen.[5] MOMC can be obtained in cultures of peripheral blood mononuclear cells (PBMC) for 7 to 10 days on fibronectin-coated plastic plates in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum as an only source of growth factors.[5] The majority of adherent cells obtained in this culture have a

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fibroblast-like morphology, which is apparently different from that of macrophages and dendritic cells. Interestingly, these cells contain progenitors capable of differentiating along several distinct non-hematopoietic lineages.

By electron microscopic examination, MOMC represent mixed features of phagocytes (primary lysosomes and cell surface projections like pseudopodia), mesenchymal cells (prominent bundles of intermediate filaments and small lipid droplets), and endothelial cells (rod-shaped microtubulated bodies). MOMC express hematopoietic and monocyte lineage markers, including CD45, CD11b, and CD14. MOMC also show expression of several stem cell markers, such as CD34 and CD105/SH2, but lack expression of CD117/c-kit and CD133. Endothelial markers CD144/VE-cadherin and vascular endothelial growth factor (VEGF) receptor type 1 (VEGFR1)/Flt-1 are present on the surface of MOMC. In addition, MOMC are positive for type I and III collagens, fibronectin, and vimentin, which are typically produced by cells of mesenchymal origin. These findings clearly show that MOMC have mixed morphologic and phenotypic features of phagocytes, mesenchymal cells, and endothelial cells. These characteristics are unique, and are not consistent with any other previously described cells derived from human peripheral blood.

MOMC appear to be originated from circulating monocytes because they are positive for monocytic markers. In fact, appearance of MOMC in PBMC cultures was completely inhibited by the depletion of $CD14^+$ monocytes. To further confirm the monocytic origin of MOMC, highly enriched $CD14^+$ monocytes that were pre-labelled with a green fluorescent dye were cultured with unlabelled $CD14^-$ PBMC (predominantly lymphocytes) on fibronectin-coated plates.[5] As expected, fluorescence-labelled cells exclusively showed a fibroblastic morphology and expressed CD34, indicating that precursors for MOMC are present within circulating $CD14^+$ monocytes. Interestingly, MOMC differentiation was not detected when monocytes were cultured alone on fibronectin or were cultured with $CD14^-$ cells on untreated plates, indicating that $CD14^-$ cells and binding to fibronectin are both required for the differentiation from monocytes to MOMC. On the other hand, the MOMC differentiation was observed when $CD14^+$ monocytes were cultured alone in the conditioned medium generated by culture of $CD14^-$ cells on fibronectin, suggesting an important role of soluble factor(s) produced by $CD14^-$ cells, rather than a cell-to-cell contact.

The number of MOMC increases during culture, but cell expansion becomes slower after the fourth passage and the cell proliferation stops beyond fifth passages, indicating that MOMC have the ability to self-replicate, but their lifespan is limited.[5] We tried hard to establish MOMC clones without any success, because of limited proliferative capacity. This feature is apparently different from stem cells.

Differentiation potentials of MOMC

MOMC can differentiate into a variety of mesenchymal cell types in specific permissive culture conditions principally developed for mesenchymal stem cells.[5] The induction treatment of MOMC *in vitro* resulted in the expression of genes and proteins specific for bone, cartilage, fat, and skeletal muscle. The differentiation of MOMC into individual mesenchymal cells followed the steps observed in mesenchymal stem cell differentiation, in terms of the timing of lineage-specific transcription factor expression. For example, expression of the myogenic transcription factor MyoD preceded the expression of skeletal muscle actin and myosin. Our finding represented the first evidence that human circulating $CD14^+$ monocytes are a source of progenitors that exhibit mesenchymal cell differentiation.

In vitro differentiation of MOMC along the cardiomyogenic lineage required a co-culture with cardiomyocytes prepared from the embryonic rat heart.[6] During the first 10 days of co-culture, the morphology of MOMC changed from spindle-shaped to round and spread out, and the majority of MOMC expressed the cardiomyocyte-specific transcription factors, such as Nkx2.5, GATA-4, eHAND, and MEF2. After 2 weeks of co-culture with rat cardiomyocytes, MOMC gradually displayed a marked increase in surface area and became multiangular morphology. Spontaneously beating MOMC were observed after 3 weeks of the co-culture, albeit at relatively low efficiency (<5% of total MOMC). These cells made contact with the surrounding rat cardiomyocytes and contracted in synchrony. At this stage, MOMC expressed cardiomyocyte-specific structural proteins, such as α-sarcomeric actinin and troponin I, with typical staining patterns of the sarcomeric structures. In addition, MOMC expressed connexin43, a protein consisting of gap junctions, and ultimately formed cell-to-cell contacts with the surrounding rat cardiomyocytes. Microinjection of the fluorescent dye into MOMC revealed coupling as determined by direct dye transfer to neighboring rat cardiomyocytes. Cytoplasmic staining of atrial natriuretic peptide (ANP), which is almost exclusively secreted by atrial cardiomyocytes, was observed in the perinuclear regions of MOMC. Expression of CD45 and CD14 was gradually down-regulated during cardiomyogenic differentiation and was lost when they started spontaneous beating. An electrophysiological study revealed that contracting MOMC showed spontaneous periodic action potentials typical of cardiac myocytes. These observations clearly indicate that a certain subset of MOMC is able to differentiate into cardiomyocytes of a mature phenotype with typical electrophysiological characteristics *in vitro*. The differentiation of

MOMC into cardiomyocytes followed the steps observed in normal differentiation, in terms of the timing of lineage-specific transcription factor expression.

Subsequently, we demonstrated that human MOMC can differentiate *in vitro* into the neuronal lineage using the similar co-culture assay using primary cultures of neuronal cells prepared from the embryonic rat brain.[7] Within 3 days of co-cultivation, the vast majority of MOMC showed nuclear expression of early neuroectodermal transcription factors, including Mash1, Ngn2, and NeuroD. These transcription factor-positive MOMC co-expressed nestin, an intermediate filament protein expressed during neurogenesis.[8] Over the next 2 weeks of the culture with rat neurons, a small population of MOMC displayed a multi-polar neuron-like morphology. MOMC expressing neurofilament had numerous axon-like processes projecting long distances and formed complex neural networks on the co-cultivated rat neurons. MOMC expressed β3-tubulin and MAP2, which are known to be preferentially expressed by axonal processes in both shaft and spine synapses. In addition, these neuron-like MOMC also exhibited nuclear expression of the neuron-specific RNA-binding protein Hu and the postmitotic neuron-specific nuclear protein NeuN. At this stage, MOMC-derived neuron-like cells lost the expression of CD45 and CD14. Again, the differentiation of MOMC into neurons followed the steps observed in normal differentiation; i.e. the expression of pro-neuronal transcription factors preceded the expression of mature neuron-specific nuclear and structural proteins. Taken together, a subset of MOMC is capable of differentiating along the neuronal lineage when placed into an appropriate environment, although their differentiation efficiency into mature neurons with typical morphologic and molecular features was very low (<5% of the total MOMC).

We recently reported that MOMC can differentiate into endothelium of a mature phenotype with typical morphologic, phenotypic, and functional characteristics.[9] MOMC treated with a combination of angiogenic growth factors for 7 days changed their morphology from spindle-shaped to caudate, and these cells had numerous rod-shaped microtubulated structures resembling Weibel-Palade bodies. Almost every MOMC expressed endothelial markers, such as CD31, CD144, VEGFR1/Flt-1, VEGFR2/KDR, Tie-2, von Willebrand factor (vWF), endothelial nitric oxide synthase (eNOS), and CD146, but expression of CD14 and CD45 was markedly down-regulated. Functional characteristics, including vWF release upon histamine stimulation and up-regulated expression of VEGF and VEGFR1 in response to hypoxia, were indistinguishable between the MOMC-derived endothelial-like cells and cultured mature endothelial cells. MOMC responded to angiogenic stimuli and promoted the formation of mature endothelial cell tubules in Matrigel® cultures. Finally, in xenogenic transplantation studies using a SCID mouse model in which syngeneic colon carcinoma cells were injected subcutaneously with or without human MOMC, co-transplantation of MOMC significantly promoted the formation of blood vessels, and more than 40% of the tumor vessel sections incorporated human endothelial cells derived from MOMC. It is of note that MOMC expanded during endothelial differentiation. These findings together indicate that human MOMC can proliferate and differentiate along the endothelial lineage in a specific permissive environment.

Multiple differentiation potentials of circulating $CD14^+$ monocytes are summarized in Fig. 1. Monocytes are long believed to be committed precursors specific for phagocytes, but our recent findings on MOMC indicate that circulating monocytes have capacity to differentiate into several distinct mesodermal and neuroectodermal lineages through differentiation into MOMC, although it is not known whether MOMC contain multipotent precursors or a group of monopotent precursors for several distinct lineages. Efficiencies of differentiation in *in vitro* cultures are greatly variable among cell lineages: i.e. high efficiency for endothelial and chondrogenic differentiation, but low efficiency for cardiomyogenic and neuronal differentiation. Differentiation from monocytes to non-phagocytic cells may not be induced during normal development, but may be readily induced in the presence of cues, such as massive tissue injury. Since the differentiation of monocytes into MOMC requires binding to fibronectin and soluble factor(s) from $CD14^-$ blood cells, circulating monocytes may encounter these signals at the site of tissue injury and inflammation. In this scenario, circulating monocytes infiltrate into the site of tissue injury and are exposed to fibronectin and soluble factor(s) produced by infiltrating inflammatory cells, resulting in their differentiation into MOMC. The MOMC subsequently differentiate into tissue-specific cells in response to organ-specific cues provided by the surrounding cells. By this process, monocytes may participate in tissue homeostasis by replacing differentiated cells lost to physiologic turnover, injury, and senescence.

Circulating monocytes as endothelial precursors

In accordance with MOMC's efficient differentiation potential into the endothelial lineage, recent accumulating evidence indicates that circulating $CD14^+$ monocytes serve as precursors for endothelial cells. Ultrastructural and immunohistochemical studies in animal models showed that monocytes accumulate within the vessel wall of growing collaterals, suggesting that these cells contribute to the process of vasculogenesis,[10,11] although some recent studies showed that bone marrow-derived hematopoietic cells are recruited to angiogenic region in response to VEGF and contribute to vasculogenesis not

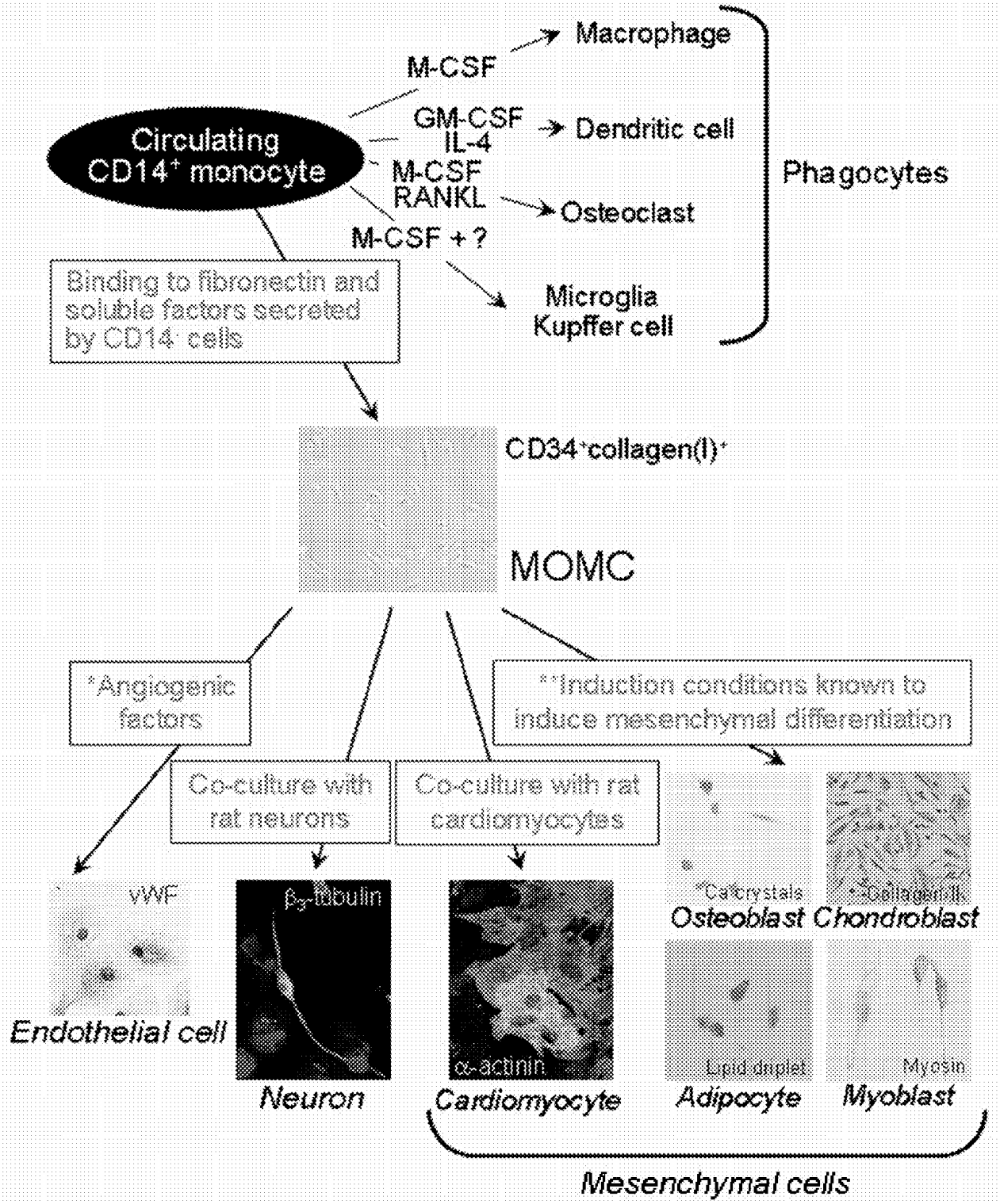

Fig. 1 Multiple differentiation potentials of circulating CD14+ monocytes through differentiation into MOMC. *Vascular endothelial growth factor, basic fibroblast growth factor (bFGF), epidermal growth factor, and insulin-like growth factor-1. **osteoblast, dexamethasone, β-glycerophosphate, and ascorbic acid; chondroblast, TGF-β1; adipocyte, dexamethasone, methyl-isobutylxatine, insulin, and indomethacin; and myoblast, pre-treatment with 5-azacitidine, following culture with horse serum, hydrocortisone, and bFGF.

being integrated as endothelial cells, but existing outside of vascular lumen.[12] Fernandez-Pujol and colleagues showed that, in the presence of angiogenic factors, monocytes differentiate into endothelial-like cells expressing endothelial lineage markers, such as vWF, CD144, CD105, and VEGFR2/KDR.[13] Schmeisser and colleagues reported that $CD14^+$ monocytes have potential to co-express endothelial lineage markers and monocytic antigens, and form tubular-like structures in three-dimensional gel.[14] In *in vivo* studies, it has been demonstrated that monocytes have the potential to be incorporated into the endothelium of co-lateral vessels and balloon-injured endothelium and to differentiate into endothelial cells.[15–17]

Asahara and colleagues first reported endothelial progenitor cells (EPC) obtained by culturing PBMC in media favoring endothelial differentiation, which were originally reported as circulating angioblasts.[18] Migration and homing of EPC to ischemic regions leads to *de novo* formation of vascular structures: a process called vasculogenesis. Later, several investigator groups found that human EPC obtained by culturing PBMC are composed predominantly of endothelial-like cells derived from circulating monocytes.[19–21] In this regard, Hur and colleagues cultured PBMC under angiogenic stimuli and identified two types of EPC.[22] "Early EPC" with spindle shape showed peak growth at 2 to 3 weeks and died after 4 weeks of the culture. On the other hand, "late EPC" representing typical endothelial cobblestone appearance became the dominant cell population after 4 weeks of the culture. Gulati *et al* reported similar findings.[23] These reports suggested that the vast majority of early EPC arise from $CD14^+$ monocytes, but late EPC develop exclusively from angioblasts within the $CD14^-$ fraction. Taken together, the majority of EPC are derived from circulating $CD14^+$ monocytes. However, MOMC were integrated into blood vessels and differentiated into endothelium *in vitro* and *in vivo* more efficiently than did freshly isolated circulating monocytes and monocytic EPC.[9]

Monocyte-derived cells with potentials to differentiate into non-phagocytes

Until now, several distinct human cell populations that are originated from circulating monocytes and have capacity to differentiate into non-phagocytes have been described. Zhao and colleagues demonstrated that pluripotent stem cells can be generated from a subset of peripheral blood monocytes by repeated stimulation with a high concentration of macrophage-colony stimulating factor and phorbol myristate acetate.[24] These spindle-shaped $CD34^+$ cells termed pluripotent stem cells (PSC) had the capacity to differentiate along several distinct cell lineages: mature macrophages by stimulation with lipopolysaccharide, T lymphocytes by stimulation with IL-2, epithelial cells by stimulation with epidermal growth factor, endothelial cells by stimulation with VEGF, neuronal cells by stimulation with nerve growth factor, and liver cells by stimulation with hepatocyte growth factor. It is interesting to mention that these authors successfully establish PSC clones from a single cell, and confirm the ability of single PSC to differentiate into distinct cell lineages, further substantiating the pluripotent nature.

On the other hand, Romagnani and colleagues have reported that monocyte-derived EPC proliferate in response to stem cell growth factors, and exhibit multi-potency, as shown by their ability to differentiate not only into endothelial cells, but also into osteoblasts, adipocytes, or neural cells.[25] In this report, the authors argue that monocytic EPC are originated from $CD34^{low}$ cells within circulating $CD14^+$ monocytes. Low expression of CD34 was not detectable by conventional flow cytometric analysis, but was detected using a highly-sensitive antibody-conjugated magnetofluorescent liposome technique. The $CD14^+CD34^{low}$ cells exhibited clonogenicity and high expression of embryonic stem cell markers, Nanog and Oct-4. Unfortunately, we were unable to reproduce isolation of $CD14^+CD34^{low}$ pluripotent cells using a highly-sensitive flow cytometric technique.

In early 1990s, Bucala and colleagues described a population of circulating cells with fibroblast properties that specifically enter sites of tissue injury.[26] This novel cell type, termed fibrocytes, was characterized by its distinctive phenotype positive for CD45, CD34, and type I collagen by its rapid entry from blood into subcutaneously implanted wound chambers. Fibrocytes contribute to scar formation and may play an important role both in normal wound repair and in pathological fibrotic responses. Fibrocytes are identified by characteristic phenotype positive for series of chemokine receptors, such as CCR7, CXCR4, and CCR2.[27,28] Fibrocytes can be enriched by culturing PBMC on tissue culture plates coated with fibronectin or type I collagen for 14 days,[26] but Abe and colleagues recently found that human circulating $CD14^+$ monocytes in the presence of T cells gives rise to fibrocytes.[29] Therefore, circulating monocytes possibly act as progenitors for fibroblasts to promote physiologic or pathologic fibrosis.

Table 1 lists human monocyte-derived cells with potentials to differentiate into non-phagocytes. These monocyte-derived cells commonly have spindle-shaped morphology and express CD45 and CD34, but have several distinct characteristics. PSC and fibrocytes are able to self-replicate and expand in long-term cultures, whereas MOMC have limited lifespan like monocytic EPC. In addition, generation of monocytic EPC and fibrocytes in PBMC cultures required either fibronectin or type I collagen, but the MOMC induction culture using

Table 1 Cultured human cell populations that are originated from circulating monocytes and have capacity to differentiate into non-phagocytes

	MOMC (Kuwana *et al.* 2003)	PSC (Zhao *et al.* 2003)	Monocytic (early) EPC	Fibrocyte (Bucala *et al.* 1994)
Morphology	Spindle shape	Spindle shape	Spindle shape	Spindle shape
CD14	+	+	+	–
CD45	+	+	+	+
CD34	+	+	+	+
Type I collagen	+	N/D	–	+
Lifespan	Limited	Unlimited	Limited	Unlimited
Cellular origin within $CD14^+$ monocytes	Not identified	Not identified	$CD14^+CD34^{low}$(?)	$CCR7^+CXCR4^+CCR2^+$
Factors required for differentiation from monocytes	Binding to fibronectin Soluble factors from $CD14^-$ blood cells	A high concentration of M-CSF and PMA	Binding to fibronectin or type I collagen Angiogenic growth factors	Binding to fibronectin or type I collagen T lymphocytes
Differentiation potential	Osteoblast Skeletal myoblast Chondrocyte Adipocyte Cardiomyocyte Endothelial cell Neuron	Macrophage T lymphocyte Epithelial cell Endothelial cell Neuron Hepatocyte	Osteoblast Adipocyte Endothelial cell Neuron	Fibroblast Myofibroblast

MOMC, monocyte-derived multipotential cells; PSC, pluripotent stem cells; EPC, endothelial progenitor cells; M-CSF, macrophage-colony stimulating factor; PMA, phorbol myristate acetate.

type I collagen instead of fibronectin failed to generate cells with multiple differentiation potentials (our unpublished data). Since cellular origins of these cell types have not been fully identified yet, circulating precursors within circulating $CD14^+$ monocytes may be different. Alternatively, distinct differentiation potentials of these primitive cells might be due to different culture conditions of the same precursors.

Summary and conclusions

We have now several lines of convincing evidence showing that circulating $CD14^+$ monocytes have the potential to differentiate into various non-phagocytes, including mesodermal and neuroectodermal lineages. In 1867, Cohnheim described that peripheral blood monocytes participate in the normal tissue renewal of various organs.[30] This investigator probably reached the right conclusion too early according to the times of science. These observations challenge the traditional view of the biology of the monocyte/phagocyte system. We believe that it will lead to further progress in the understanding of the differentiation potential of monocytes and the roles they play in the physiology of health and disease. On the other hand, cell replacement therapy may become a useful approach for repairing the lethally damaged tissue. A variety of cell types have been proposed as transplantable cells for tissue regenerative therapy, but the multi-potentiality of circulating cells has been underestimated to date. Cellular therapy using primitive cells derived from circulating monocytes has considerable advantages over the currently proposed strategies using tissue-specific stem cells and embryonic stem cells, since circulating monocytes would be a relatively obtainable source of autologous cells. Among these primitive cells, MOMC can differentiate into a variety of cell types across lineages. However, further *in vivo* studies evaluating whether the transplantation of monocyte-derived primitive cells such as MOMC promote regeneration in the damaged tissues are necessary to confirm their potential use in cell therapy.

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